Amendments to the Specification:

Please replace the paragraph beginning on page 1, line 21, with the following amended paragraph:

Four different groups of mutant genes are known to cause early-onset familial AD (FAD): V642I/F/G APP (the number ~~represnts~~ **represents** the position in APP_{695}, an APP consisting of 695 amino acids); K595N/M596L APP (NL-APP); presenilin (PS)-1 mutants; and PS-2 mutants (Shastry, B.S. and Giblin, F.J. (1999) Brain Res. Bull. 48, 121-127). Yamatsuji et al. suggested that these FAD genes might cause cell death of neurons, based on the observation on nerve cell line F11 wherein three V642 type mutant cDNA of APP was transiently expressed (Yamatsuji, T. et al. (1996) Science 272, 1349-1352). The result was also confirmed by experiments that used primary cultured neurons and other nerve cell lines (Zhao, B. et al. (1997) J. Neurosci. Res. 47, 253-263; Luo, J. J. et al. (1999) J. Neurosci. Res. 55, 629-42). Further, Wolozin et al. revealed that FAD-linked mutant N141I PS-2 significantly enhances cell mortality in PC12 cells, and that FAD-linked mutant PS-1 induces apoptosis of T lymphocytes (Wolozin, B. et al. (1996) Science 274, 1710-1713; Wolozin, B. et al. (1998) Neurobiol. Aging 19, S23-27). Furthermore, regarding PS-1, enhanced sensitivity to neuronal death induced by Aβ addition and/or trophic factor deficiency due to the expression of PS-1 mutant (Guo, Q. et al. (1996) Neuroreport 8, 379-83; Zhang, Z. et al. (1998) Nature 395, 698-702; Guo, Q. et al. (1999) Proc. Natl. Acad. Sci. U.S.A. 96.,4125-30); and enhanced sensitivity to neuronal death by trophic factor deficiency in cultured cortical neurons, derived from transgenic rats overexpressing wild-type PS-1, compared with those in non-transgenic controls (Czech, C. et al. (1998) Neuroscience 87, 325-36); and such have been repeatedly observed. Although it is controversial whether the mutant PS-1 is a stimulation factor of neuronal death or has no effect on neuronal death (Weihl, C. C. et al. (1999) J. Neurosci. 19, 5360-9; Bursztajn, S. et al. (1998) J. Neurosci. 18, 9790-9), it is highly possible that all of the four types of known FAD genes (V642-type mutant APP, NL-APP, PS-1 mutant, and PS-2 mutant) induce neuronal death or amplify the vulnerability of neurons to other cell death stimuli under certain conditions. Therefore, finding molecules that

suppress AD gene-induced cell death observed in neurons is suggested to be the most important key for developing methods to treat AD.

Please replace the paragraph beginning on page 7, line 27, with the following amended paragraph:

More specifically, a cDNA library was constructed from the brain of Alzheimer's disease (AD) patient, and was transfected into the F11/EcR/V642I cells mentioned above. Then a death trap screening operation was repeatedly performed to select cells that survived neuronal death induced by V642I APP. As a result, the present inventor succeeded in identifying a novel gene that protect cells against neuronal death induced by V642I APP. It was revealed that the clone, dubbed Humanin (HN) cDNA, encoding a novel polypeptide of 24-amino acids, suppresses neuronal death associated with AD. That is, the clone suppressed neuronal death induced by all of the known types of early-onset familial AD genes [V642I APP, K595N/M596L APP, M146L presenilin (PS)-1, and N141I PS-2] and by Aβ1-43. In contrast, the clone had no effect on neurotoxicity of polyglutamine repeat Q79, associated with Huntington's disease (HD)/spinocerebellar ataxia (SCA); and mutants of Cu/Zn-dependent superoxide dismutase (SOD1), associated with amyotrophic lateral sclerosis (ALS). HN mRNA was mainly produced in several organs other than the central nervous system. Transfection of HN cDNA into neurons led to transcription and production of expected peptides, which peptides were secreted into the culture medium up to a level of about 10 μM. The culture supernatant was enough active to demonstrate significant protection of cells from neuronal death induced by V642I APP. Synthetic HN polypeptide also showed neuroprotective action with similar dose-response properties against the four types of AD genes, and its suppression was maximal at 1 to 10 μM. Polypeptides expressed within neurons from a cDNA encoding an HN derivative, lacking secretion ability, failed to protect neurons from cell death. However, the same polypeptide synthesized and added to the culture medium showed protective action, which results indicate that the HN polypeptide acts from outside of the cell. Cys at position 8 and Ser at position 14 were found to be important according to an experiment detecting the activity of polypeptides

with modified structure. C8A substitution completely deprived the polypeptide of the cell death rescue activity. On the other hand, S14G substitution remarkably enhanced the rescue activity of the polypeptide. S14G HN polypeptides (HNG) showed complete protective action against all of the four types of FAD genes at low nanomolar concentrations (1 to 10 nM). Anti-AD activity of HN was also observed in primary cultured cortical neurons. Specifically, μM levels of HN and nM levels of S14G derivatives (HNG) protected cells form cell death and cell damage caused by Aβ, whereas C8A (HNA) lacked such activity. Furthermore, upon analysis of detailed structure-function relationship, amino acids from Pro at position 3 to Pro at position 19 were identified to be important for neuroprotective function, and among them, seven residues were identified as essential residues for the activity. In addition, C8 amino acid of S14G HN polypeptide (HNG) could be substituted with basic amino acids, such as His, Arg, or Lys, while maintaining its anti-AD activity. Furthermore, the ~~presnt~~ **present** inventor succeeded to further enhance the neuroprotective action by introducing two amino acid mutations to the S14G HN polypeptide. Based on these findings, it is possible to develop polypeptides that have higher activity and which are suited for biological administration. These polypeptides open a new path to develop therapeutic drugs for AD, and at the same time, are expected to contribute greatly to the development of AD therapy aiming at protection of neurons from cell death.

Please replace the paragraph beginning on page 7, line 27, with the following amended paragraph:

Further, the present invention demonstrated that addition of a FLAG tag (DYKDDDDK) **(residues 27-34 of SEQ ID NO:6)** to the C-terminus of Humanin does not affect the neuroprotective action thereof (Example 3). Furthermore, even when the four C-terminal amino acids (KRRA) **(residues 21-24 of SEQ ID NO:5)** of Humanin were substituted with other amino acids, a neuroprotective action equivalent to that of the original Humanin was present in the substituted polypeptide (Example 6). These facts demonstrate that polypeptides with equivalent or higher neuroprotective action to can be prepared by introducing mutations to the amino acid sequence of Humanin, HNG, AGA-HNG, and substituted forms thereof, wherein the C8 is substituted with a basic amino acid.

Please replace the paragraph beginning on page 9, line 2 with the following amended paragraph:

The polypeptide of the present invention includes polypeptides that suppress neuronal death associated with Alzheimer's disease (AD) and having an amino acid sequence consisting of Formula (I):

Pro-Xn_1-(Cys/bXaa)-(Leu/Arg)-Xn_2-Leu-Thr-(Gly/Ser)-Xn_3-Pro (I) **(SEQ ID NO:61)**.

Herein, "Cys/bXaa" indicates Cys or a basic amino acid; "(Leu/Arg)" indicates Leu or Arg; "(Gly/Ser)" indicates Gly or Ser; and Xn_1, Xn_2, and Xn_3 independently indicate arbitrary amino acids not more than 10 residues, respectively. A polypeptide that has the amino acid sequence as above may be also expressed as:

Pro-$(Xaa)_{1-10}$-(Cys/bXaa)-(Leu/Arg)-$(Xaa)_{1-10}$-Leu-Thr-(Gly/Ser)-$(Xaa)_{1-10}$-Pro **(SEQ ID NO:61)**

(II)

(wherein Xaa indicates an arbitrary amino acid; "$(Xaa)_{m-n}$" indicates m to n residues of arbitrary amino acids; "bXaa" indicates a basic amino acid; "Cys/bXaa" indicates Cys or a basic amino acid; "(Leu/Arg)" indicates Leu or Arg; and "(Gly/Ser)" indicates Gly or Ser).

Please replace the paragraph beginning on page 9, line 18 with the following amended paragraph:

Basic amino acids refer to amino acids in which its R group (side chain) is positively charged at pH7.0. Examples of natural basic amino acids include Arg, Lys, and His. The amino acid sequences of a polypeptide of this invention that has Arg, Lys, or His as the basic amino acids can be represented, for example, as:

Pro-Xn_1-(Cys/Arg/Lys/His)-(Leu/Arg)-Xn_2-Leu-Thr-(Gly/Ser)-Xn_3-Pro **(SEQ ID NO:62)**

(III)

(wherein "(Cys/Arg/Lys/His)"indicates Cys, Arg, Lys, or His; "(Leu/Arg)" indicates Leu or Arg; "(Gly/Ser)" indicates Gly or Ser; and Xn_1, Xn_2, and Xn_3 independently indicate arbitrary amino acids not more than 10 residues, respectively). Herein, Arg and Lys are particularly preferable as the basic amino acid at this position.

Please replace the paragraph beginning on page 10, line 9, with the following amended paragraph:

Preferably, the sequence of Xn_1 includes, for example, sequences consisting of (Arg/Ala)-(Gly/Ala)-(Phe/Ala)-(Ser/Ala) **(SEQ ID NO:96)**, and sequences with conservative substitution thereof. Herein, for example, "Arg/Ala" indicates Arg or Ala ("/" indicates that it is either one of the residues; the same is indicated throughout the description herein). Examples of such sequences include Arg-Gly-Phe-Ser **(SEQ ID NO:63)**, Ala-Gly-Phe-Ser **(SEQ ID NO:64)**, Arg-Ala-Phe-Ser **(SEQ ID NO:65)**, Arg-Gly-Ala-Ser **(SEQ ID NO:66)**, Arg-Gly-Phe-Ala **(SEQ ID NO:67)**, and so on. Other examples include Arg-Gly-Ala-Ala **(SEQ ID NO:68)**, Arg-Ala-Phe-Ala **(SEQ ID NO:69)**, Arg-Ala-Ala-Ser **(SEQ ID NO:70)**, Arg-Ala-Ala-Ala **(SEQ ID NO:71)**, Ala-Gly-Phe-Ala **(SEQ ID NO:72)**, Ala-Gly-Ala-Ser **(SEQ ID NO:73)**, Ala-Gly-Ala-Ala **(SEQ ID NO:74)**, Ala-Ala-Phe-Ser **(SEQ ID NO:75)**, Ala-Ala-Phe-Ala **(SEQ ID NO:76)**, Ala-Ala-Ala-Ser **(SEQ ID NO:77)**, Ala-Ala-Ala-Ala **(SEQ ID NO:78)**, and such. Conservative substitution can be exemplified by substitution within a group of amino acids, corresponding to conservative substitution, which will be described later. On the other hand, the sequence of Xn_2 preferably includes, for example, sequences consisting of (Leu/Ala)-(Leu/Ala), and sequences with conservative substitution thereof. Such sequences include Leu-Leu, Ala-Leu, Leu-Ala, and such. Ala-Ala can be also exemplified as such sequences. Furthermore, the sequence of Xn_3 preferably includes, for example, sequences consisting of (Glu/Ala)-(Ile/Ala)-(Asp/Ala)-(Leu/Ala) **(SEQ ID NO:79)**, and sequences with conservative substitution thereof. Such examples include Glu-Ile-Asp-Leu **(SEQ ID NO:80)**, Ala-Ile-Asp-Leu **(SEQ ID NO:81)**, Glu-Ala-Asp-Leu **(SEQ ID NO:82)**, Glu-Ile-Ala-Leu **(SEQ ID NO:83)**, Glu-Ile-Asp-Ala **(SEQ ID NO:84)**, and so on. Other examples are Glu-Ile-Ala-Ala **(SEQ ID NO:85)**, Glu-Ala-Asp-Ala **(SEQ ID NO:86)**, Glu-Ala-Ala-Leu **(SEQ ID NO:87)**, Glu-Ala-Ala-Ala **(SEQ ID NO:88)**, Ala-Ile-Asp-Ala **(SEQ ID NO:89)**, Ala-Ile-Ala-Leu **(SEQ ID NO:90)**, Ala-Ile-Ala-Ala **(SEQ ID NO:91)**, Ala-Ala-Asp-Leu **(SEQ ID NO:92)**, Ala-Ala-Asp-Ala **(SEQ ID NO:93)**, Ala-Ala-Ala-Leu **(SEQ ID NO:94)**, Ala-Ala-Ala-Ala **(SEQ ID NO:95)**, and so on. The sequences of Xn_1, Xn_2, and Xn_3 may be selected from arbitrary combinations.

Please replace the paragraph beginning on page 10, line 9, with the following amended paragraph:

Such DNA includes, probes and primer for detecting or amplifying DNAs or RNAs encoding a peptide of the invention; as well as nucleotide and nucleotide derivatives (for example, antisense oligonucleotides, DNAs encoding ~~ribozymser~~**ribozymes**, and such) for suppressing the expression of a polypeptide of this invention. When used as a primer, such a DNA is complementary at the 3'-end, and restriction enzyme recognition sequences or tags can be added to the 5'-end.

Please replace the paragraph beginning on page 27, line 25, with the following amended paragraph:

Figure 1 depicts a schematic illustration of the region in Humanin cDNA clone **(SEQ ID NO:4)** that encodes a polypeptide that antagonizes cell death caused by V642I APP.

Please replace the paragraph beginning on page 34, line 36 with the following amended paragraph:

Figure 21 depicts bar graphs demonstrating the result of a detailed analysis of the structure/function relationship regarding the protective activity of HN. The effect of HN-truncated derivatives on V642I APP-induced neuronal death is demonstrated. As indicated in Figure 9, F11 cells were transfected with V642I APP cDNA in the presence or absence of each of the synthetic HN derivatives; and 72 hours later, cell death was measured by trypan blue exclusion assay. The top panel on the left demonstrates the result of examination on the N-terminus truncated HNs **(SEQ ID NOS:5, and 12-19)**, which sequences are indicated in the panel below. The top panel on the right demonstrates the result of examination on the C-terminus truncated polypeptides of ΔN2 HN **(SEQ ID NOS:20-23)**, which sequences are indicated in the panel below. Mean ±S.D. values of three independent experiments are indicated. The results obtained in the experiments indicated in the panel are summarized in the lower panel.

Please replace the paragraph beginning on page 35, line 30, with the following amended paragraph:

Figure 23 depicts bar graphs demonstrating the effect of Ala-substituted (Ala-scanned) HNG-17 **(SEQ ID NO:24)** on neuronal death caused by four different types of FAD

genes and Aβ1-43. Primary cultured neurons were treated with 25 μM Aβ1-43 (top panel on the left), or F11 cells were transfected with V642I APP, NL-APP, M146L PS-1 or N141I PS-2 cDNA (other panels) in the presence or absence of 10 nM Ala-substituted HNG-17; and 72 hours later, cell death was measured by trypan blue exclusion assay. Cell death antagonizing effect of each Ala-substituted HNG-17 was determined. Substituted residues are indicated at the bottom of the graphs. Amino acid sequences of each Ala-~~substited~~**substituted** form are indicated in order from SEQ ID NO: 25 to 41. For example, in the top panel on the left, the primary cultured neurons incubated for 72 hours with 25 μM Aβ1-43 in the presence of 10 nM **ARGFSCLLLLTGEIDLP** ~~ARGFSCLLLLTGEUDLP~~ (the underlined A is substituted from P) (SEQ ID NO: 25) showed a cell mortality of 75.3±4.4% (mean ±S.D. of three independent experiments), which was comparable to the cell mortality of neurons incubated with Aβ1-43 (76.1±4.7%). When neurons were incubated with 25 μM Aβ1-43 in the presence of 10 nM HNG or HNG-17, cell mortality was 29.3±0.9% or 28.8±1.3% respectively, which was comparable to the basal cell mortality (30.0±1.6%). The results indicated as "no T" are results of cells untransfected with FAD genes; "vec" of cells transfected with an empty vector; and "no" of cells untreated with polypeptides. Mean ±S.D. values of three independent experiments are indicated in the graphs.

Please replace the paragraph beginning on page 37, line 16, with the following amended paragraph:

Figure 25 depicts graphs demonstrating the effect of AGA-HNG (SEQ ID NO: 60) on neuronal death induced by the four different types of FAD genes and Aβ1-43. ~~Priamry~~ **Primary** cultured neurons were treated with 25 μM Aβ1-43 in the absence or presence at various concentrations of AGA-HNG (panel B), or F11 cells were transfected with V642I APP, NL-APP, M146L PS-1, or N141I PS-2 cDNA (panels C to F); and 72 hours later, cell death was measured by trypan blue exclusion assay. In the primary cultured neuron experiment using Aβ, various concentrations of HNG **(SEQ ID NO:8)** were used to perform similar experiments as controls. The result of untransfected cells is indicated as "no T"; and the result of cells

transfected with the empty vector as "pcDNA". Mean ±S.D. values of three independent experiments are indicated in the graphs.

Please replace the paragraph beginning on page 48, line 32, with the following amended paragraph:

The present inventor further examined whether the cell death suppressive action of sHN is dependent on the specific primary structure (Figure 9). A complete antagonizing effect on cell death induced by V642I APP could be observed at a concentration of 10 nM or less with S14G (MAPRGFSCLLLLTGEIDLPVKRRA: the underlined G replaces S; called HNG) (SEQ ID NO: 8) as the polypeptide, and IC_{50} of the polypeptide was about 100 pM. In contrast, C8A HN polypeptide (MAPRGFSALLLLTSEIDLPVKRRA: underlined A replaces C; called HNA) (SEQ ID NO: 9) did not significantly suppress cell death induced by V642I APP at concentrations up to 100 μM. The importance of Cys at position 8 was also suggested from the result obtained using an HN dimer (C8-C8 HN), bound through Cys at position 8. The antagonizing action level of C8-C8 HN was in between those of the original HN and HNA. On the contrary, a derivative wherein the HN C-terminal KRRA **(residues 21-24 of SEQ ID NO:9)** was substituted with AAAA **(residues 21-24 of SEQ ID NO:10)** ~~(SEQ ID NO: 10)~~ indicated similar functional activity to the original HN polypeptide. These results indicate that the primary structure has a fundamental role in the suppression activity of HN, and that particular amino acid residues have a predetermined role.

Please replace the paragraph beginning on page 49, line 17, with the following amended paragraph:

Next, the effect of sHN, synthetic HNG (sHNG), and synthetic HNA (sHNA) on cell death induced by other FAD genes, more specifically, those induced by NL-APP, M146L PS-1, and N141I PS-2 was investigated. As indicated in Figure 10, the original sHN demonstrated similar dose-responsiveness on cell death induced by any of the three FAD genes, and blocked neuronal death induced by the FAD genes at a concentration of 1 μM. Up to a concentration of 100 μM, sHNA did not antagonize cell death by any of the FAD genes. In

contrast, sHNG completely suppressed cell death caused by any of the FAD genes at a concentration of 10 nM or less. This indicates that the action of HN is enhanced 100 to 1000 fold by S14G substitution. Taking the action of sHNG on cell death induced by V642I APP (Figure 9) ~~togeter~~**together**, sHNG at a concentration of 10 nM or less, completely antagonizes neuronal death induced by all of the four different types of FAD genes.

Please replace the paragraph beginning on page 55, line 22, with the following amended paragraph:

Detailed relationship between the primary structure and protective activity of HN was investigated. Figure 21 demonstrates the result of analysis examining whether a truncated HN polypeptide antagonizes V642I APP. F11 cells were transfected with V642I APP cDNA and were cultured in the presence of 10 μM HN derivative. Hardly any effect on cell protective activity was observed by the deletion of two N-terminal residues from the HN polypeptide in the system. On the other hand, activity of HN disappeared by a deletion of three residues from its N-terminus. This suggests that although Met-Ala at the N-terminal end is not necessary, the third Pro is essential for the activity. According to a similar experiments on the deletion of the C-terminus, as demonstrated in Figure 21, Val-Lys-Arg-Arg-Ala **(residues 20-25 of SEQ ID NO:42)** at the C-terminal end is not necessary whereas the nineteenth Pro was essential for maintaining the complete protective activity of HN. Therefore, the minimum region required for maximum activity is from Pro3 to Pro19, which was dubbed HN-17 (SEQ ID NO: 21).

Please replace the paragraph beginning on page 58, line 17, with the following amended paragraph:

F11 cells in their original form, or F11 cells transfected with V642I-APP cDNA (1 μg) were treated with any one of the mutated HNG polypeptides (10 nM), which Cys at position 8 (C8) was substituted with one of the 19 other possible amino acid residues. 72 hours after transfection initiation, cell mortality was measured by trypan blue exclusion assay. The results ~~demonsatrated~~**demonstrated** that polypeptides, whose C8 was substituted with basic

amino acids, such as His (SEQ ID NO: 46), Lys (SEQ ID NO: 48), or Arg (SEQ ID NO: 54), indicated significant suppression activity on neuronal death (Figure 24, top panel).

Please replace the paragraph beginning on page 59, line 24, with the following amended paragraph:

Enhancement of the HNG action by mutation of HNG (S14G HN) was verified. According to an experiment, examining the neuroprotective action of HNGs wherein multiple amino acid residues are substituted with other amino acids, polypeptides with higher rescue activity compared to HNG were ~~obatined~~**obtained** by mutating two positions, R4A/F6A (SEQ ID NO: 60). The polypeptide, dubbed AGA-HNG, not only completely rescued cell death of nerve cell line induced by FAD genes at a concentration as little as 0.1 nM, but completely rescued cell death of primary cultured neurons by Aβ at a concentration of 0.3 nM (Figure 25). Arg and Phe at position 4 and 6 of HNG, respectively, are positions which are cleaved by trypsine-like protease and chymotrypsin-like protease, respectively (see Figure 25A). Thus, it is likely that R4A/F6A substitution of HNG enhances resistance to degradation. A remarkable high activity for AGA-HNG is implied by the fact that AGA-HNG detoxifies the neurotoxicity of Aβ of high concentration, a 100000-fold higher concentration than AGA-HNG. Anti-AD agent that has such a broad spectrum with high neuroprotective action has not been reported so far. Application of AGA-HNG or derivatives thereof to chemotherapy of AD is expected.

Please replace the sequence listing filed with the application on March 18, 2002, with the paper copy of the sequence listing, comprising pages 1-29, enclosed herewith.